IN THE CLAIMS

On page 76 of the copy of the publication, the amendments to the listing of claims serves to replace prior versions of claims from its related international application.

Listing of claims

1. (Currently amended) An assembly comprising a gas-filled microvesicle bearing a first overall net charge and a component associated ~~to~~ with said microvesicle wherein said component bears a second overall net charge opposite in sign to said first net charge and comprises a targeting ligand, a diagnostic agent or any combination thereof, and a biocompatible surface active agent.

2. An assembly according to claim 1 wherein said targeting ligand is selected from proteins, antibodies, antibody fragments, receptor molecules, receptor binding molecules, glycoproteins, lectins, peptides, oligopeptides, polypeptides, peptidomimetics, saccharides, polysaccharides, vitamins, steroids, steroid analogs, hormones, cofactors, bioactive agents, genetic material, nucleosides, nucleotides and polynucleotides.

3. An assembly according to claim 1 wherein said diagnostic agent is selected from magnetite nanoparticles, iodinated compounds and paramagnetic ion complexes.

4. (Currently amended) An assembly according to claim 1 wherein said component associated ~~to~~ with said gas-filled microvesicle further comprises a bioactive agent.

5. An assembly according to claim 1 further comprising at least a second component having an overall net charge opposite in sign to said first net charge and comprising a bioactive agent.

6. An assembly according to claim 1 further comprising at least a second component having an overall net charge equal in sign to the charge of the microvesicle.

7. An assembly according to claim 6 wherein said second component comprises a targeting ligand, a diagnostic agent, a bioactive agent or any combination thereof.

8. (Currently amended) An assembly according to ~~any of the preceding claims~~ claim 1, wherein said component associated ~~to~~ with said gas-filled microvesicle has a diameter of 300 nm or less.

9. An assembly according to claim 1 wherein said biocompatible surface active agent is an emulsifying agent, a dispersing agent or a mixture thereof.

10. An assembly according to claim 1 wherein said biocompatible surface active agent is an amphiphilic material.

11. An assembly according to claim 1 wherein said biocompatible surface active agent is selected among (C_2-C_{10}) organic acids, organic fatty acids comprising a (C_{12}-C_{24}) aliphatic chain, pharmaceutically acceptable salts thereof, esters thereof with polyoxyethylene; polyionic (alkali) salts; organic amines; amides; quaternary amine salts; aminoacids; phospholipids; ; esters of mono- or oligo-saccharides with (C_{12}-C_{24}), organic fatty acids; organic sulfonates; perfluoroorganic acids; polymeric surfactants; and mixtures thereof.

12. An assembly according to claim 1 wherein the ratio between the number of charges per mole of microvesicles and the number of charges per mole of the second component is from about 10:1 to about 1:10.

13. An assembly according to claim 12 wherein said ratio is of about 3:1 or less.

14. An assembly according to claim 12 wherein said ratio is of about 2:1 or less.

15. An assembly according to claim 12 wherein said ratio is of about 3:2 or less.

16. An assembly according to claim 1 wherein said microvesicle is a microbubble stabilized by an envelope comprising an amphiphilic film-forming compound or a microballoon having a material envelope.

17. (Currently amended) An assembly according to claim ~~15~~ 16 wherein said amphiphilic film-forming compound comprised in the envelope stabilizing the microbubble is a phospholipid.

18. An assembly according to claim 16 wherein said envelope comprises a phospholipid or a lipid bearing a positive or negative net charge.

19. An assembly according to claim 16 wherein the material envelope of said microballoon comprises a polymeric material, a proteinaceus material, a water insoluble lipid or any combination thereof.

20. An assembly according to claim 16 wherein the material envelope of said microballoon comprises a ionic biodegradable polymers.

21. An assembly according to claim 16 wherein the material envelope of said microballoon further comprises a phospholipid or a lipid bearing a positive or negative net charge.

22. An assembly according to claim 18 or 21 wherein said phospholipid or lipid is selected from phosphatidylserine derivatives, phosphatidic acid derivatives, phosphatidylglycerol derivatives, polyethyleneglycol modified phosphatidylethanolamines, ethylphosphatidylcholine derivatives and the respective lyso-forms; cholic acid salts; deoxycholic acid salts; glycocholic acid salts; (C_{12}-C_{24}) fatty acid salts thereof; alkylammonium salts comprising at least one (C_{10}-C_{20}) alkyl chain; tertiary or quaternary ammonium salts comprising at least one (C_{10}-C_{20}) acyl chain linked to the nitrogen atom through a (C_3-C_6) alkylene bridge; and mixtures thereof.

23. (Currently amended) An assembly according to claim 1, ~~4, 5, 6 or 7~~ wherein said component associated with ~~to~~ with microvesicle is a micelle.

24. An assembly according to claim 23 wherein said micelle comprises a polyethyleneglycol modified phospholipid; an alkylammonium salt comprising at least one (C_{10}-C_{20}) alkyl chain; a tertiary or quaternary ammonium salt comprising at least one (C_{10}-C_{20}) acyl chain linked to the nitrogen atom through a (C_3-C_6) alkylene bridge; a (C_{12}-C_{24}) fatty acid salt; a polymeric surfactant; or mixtures thereof.

25. An assembly according to claim 23 wherein said micelle comprises a (C_{12}-C_{24}) fatty acid di-esters of phosphatidylcholine, ethylphosphatidylcholine, phosphatidylglycerol, phosphatidic acid, phosphatidylethanolamine, phosphatidylserine or sphingomyelin.

26. An assembly according to claim 23 wherein said micelle comprises a phospholipid or a lipid bearing a positive or negative net charge, or a polymeric ionic surfactant.

27. An assembly according to claim 26 wherein said phospholipid or lipid is selected from phosphatidylserine derivatives, phosphatidic acid derivatives, phosphatidylglycerol derivatives, polyethyleneglycol modified phosphatidylethanolamines, ethylphosphatidylcholine derivatives and the respective lyso-forms; cholic acid salts; deoxycholic acid salts; glycocholic acid salts; (C_{12}-C_{24}) fatty acid salts thereof; alkylammonium salts comprising at least one (C_{10}-C_{20}) alkyl chain; tertiary or quaternary

ammonium salts comprising at least one (C_{10}-C_{20}) acyl chain linked to the nitrogen atom through a (C_3-C_6) alkylene bridge; and mixtures thereof.

28. (Currently amended) An assembly according to claim 1 wherein said component associated ~~to~~ with said microvesicle is a colloidal nanoparticle.

29. (Currently amended) An assembly according to claim 1 wherein said component associated ~~to~~ with said microvesicle is a solid polymeric nanoparticle.

30. (Currently amended) An aqueous suspension of a physiologically acceptable liquid comprising an assembly according to any one of ~~the~~ claims 1~~to 29~~ , 4 or 23.

31. (Currently amended) An assembly according to ~~any of the~~ claim[[s]] 1 ~~to 29~~, wherein an aqueous suspension of said assembly in a pharmaceutically acceptable carrier shows a ζ-potential which is decreased of at least 50% in absolute value with respect to the ζ-potential of an aqueous suspension in the same carrier of the gas-filled microvesicles forming said assembly.

32. An assembly according to claim 31 wherein said ζ-potential is decreased of at least 75% in absolute value.

33. An assembly according to claim 31 wherein said ζ-potential is decreased of about 100% or more in absolute value.

34. A pharmaceutical kit which separately comprises:
a) a gas-filled microvesicle, or a precursor thereof, bearing a first overall net charge as a first component;
b) a second component, or a precursor thereof, associable with said microvesicle bearing a second overall net charge opposite in sign to said first net charge, said associated component comprising a targeting ligand, a diagnostic agent or any combination thereof.

35. A pharmaceutical kit according to claim 34 further comprising a pharmaceutically acceptable liquid carrier.

36. A pharmaceutical kit according to claim 35 wherein said first and second components are in the form of separate freeze-dried preparations.

37. A pharmaceutical kit which comprises:

a) a gas-filled microvesicle, or a precursor thereof, bearing a first overall net charge as a first component;

b) a second component, or a precursor thereof, associated with said microvesicle bearing a second overall net charge opposite in sign to said first net charge, said associated component comprising a targeting ligand, a diagnostic agent or any combination thereof, and a biocompatible surface active agent.

38. (Currently amended) A ~~M~~method for preparing an assembly according to ~~any of the previous~~ claim[[s]] 1 ~~to 26~~, which comprises admixing a preparation comprising gas-filled microvesicles or a precursor thereof with a preparation comprising a component or a precursor thereof to be associated ~~to~~ with said microvesicles.

39. (Currently amended) A ~~M~~method according to claim 38 which comprises:

1) preparing a first aqueous suspension comprising a gas-filled microvesicle;

2) preparing a second aqueous suspension comprising a component to be associated with said gas-filled microvesicle;

3) admixing said two suspensions, to obtain an aqueous suspension comprising said assembly.

40. (Currently amended) A ~~M~~method according to claim 38 which comprises:

1) preparing a first aqueous suspension comprising a gas-filled microvesicle;

2) freeze-drying said suspension, to obtain a first lyophilized product;

3) preparing a second suspension comprising a component to be associated with said gas-filled microvesicle;

4) freeze-drying said suspension, to obtain a second lyophilized product;

5) reconstituting said first and said second lyophilized product with a physiologically acceptable aqueous carrier in the presence of a gas, to obtain an aqueous suspension comprising the assembly.

41. (Currently amended) A ~~M~~method according to claim 40, wherein step 5) comprises the steps of:

a) reconstituting the second lyophilized product with a physiologically acceptable aqueous carrier to obtain a suspension comprising the component to be associated to the gas-filled microvesicle; and

b) reconstituting the first lyophilized product with said suspension in the presence of a gas.

42. (Currently amended) A ~~M~~method according to claim 38 which comprises:

1) preparing an aqueous emulsion comprising an organic solvent, a phospholipid and a lyoprotecting agent;

2) preparing an aqueous suspension comprising a component to be associated with a gas-filled microvesicle;

3) admixing said aqueous suspension with said aqueous emulsion; and

4) freeze drying the mixture to remove the water and the organic solvent, to obtain a lyophilized product comprising said assembly.

43. (Currently amended) A ~~M~~method for preparing an assembly ~~of claims 6 or 7 which~~ comprising a gas-filled microvesicle bearing a first overall net charge and a component associated with said microvesicle wherein said component bears a second overall net charge equal in sign to said first net charge and comprises a targeting ligand, a diagnostic agent or any combination thereof, and a biocompatible surface active agent, wherein the method comprises admixing [[a]] the second component~~, bearing an overall net charge equal in sign with respect to the charge of the gas filled microvesicles, to~~ with the assembly obtained according to ~~any of the~~ claim[[s]] 38 ~~to 42~~.

44. (New) An assembly according to claim 23 wherein said component associated with said gas-filled microvesicle further comprises a bioactive agent.

45. ~~44.~~ (Currently amended) ~~Use of~~ A pharmaceutically active formulation comprising an assembly according to any one of ~~the~~ claims 1 ~~to 29 for preparing a~~, 4, 23, or 44.

46. ~~45.~~ (Currently amended) A method for ultrasound diagnostic imaging which comprises administering a contrast-enhancing amount of an aqueous suspension of an assembly according to any one of ~~the~~ claims 1 ~~to 29~~, 4, 23, or 44.

47. ~~46.~~ (Currently amended) A method of therapeutic treatment which comprises administering a therapeutically-effective amount of an aqueous suspension of an assembly as defined in any one of claims 4, ~~or~~ 5, or 44.

48. (New) A method for preparing an assembly comprising a gas-filled microvesicle bearing a first overall net charge and a component associated with said microvesicle wherein said component bears a second overall net charge equal in sign to said first net charge and comprises a targeting ligand, a diagnostic agent, a bioactive agent or any combination thereof, and a biocompatible surface active agent, wherein said method comprises admixing the second component with the assembly obtained according to claim 38.

49. (New) An aqueous suspension of a physiologically acceptable liquid comprising an assembly according to claim 44.